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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of
Yoshikatsu KODAMA et al.
Serial No. 10/519,536
Filed: December 28, 2004
For: ANTI-CHICKEN COCCIDIOSIS COMPOSITION

Atty. Docket No.: 2004_2037A
Art Unit: 1645
Examiner: Lynetter F. Smith

DECLARATION PURSUANT TO 37 C.F.R.1.132

1. I, Yoshikatsu KODAMA, do hereby declare as follows:

I had Ph.D. from University of Tokyo in 1978. Since April, 1978, I have been employed by GHEN Corporation. I have a full knowledge of the present invention and cited references.

2. In order to demonstrate the patentability of the present invention, the following experiment was carried out.

The animal used in this evaluation was "Cobb" which is a chicken breed. A broiler feed comprising Chick Start™ in an amount of 500 g / 1t was administered to pathogen free baby chicks until the chicks became 18 days old. Then a broiler feed comprising Chick Start™ in an amount of 250 g/ 1t was administered to the chicks until shipping. Chick Start™ is a product of GHEN Corporation which comprises $6.4 \times 10^7 \sim 1.3 \times 10^8$ / 1kg (ELISA Titer) of the anti-chicken coccidiosis antibody of the present invention. As a positive control, a broiler feed comprising Salinomycin in an amount of 100g / 1t was administered to baby chicks until shipping. Salinomycin has been widely used as an anti- coccidiosis agent. The result is shown in Table 1.

Table 1

Agent administered	Farm	Chicks receipt	Chicks shipped	Rate of maturity (%)	Average weight (g)	Feed conversion rate
Antibody of the invention	A	5100	4879	95.7	2.363	1.929
	B	5400	5110	94.6	2.474	1.929
	C	5900	5782	98	2.511	1.972
Salinomycin (positive control)	A	6750	6427	95.2	2.276	2.188
	B	6300	5971	94.8	2.149	2.188
	C	5550	5269	94.9	2.52	1.986

The farms were contaminated with E acerubulina, E. tenella and E. maxima. Therefore, Chicks would be infected with the Eimeria species and would be affected with coccidiosis without agents such as Salinimicin. When Chicks are affected with coccidiosis, average weight and rate of maturity will greatly decrease, as a result, productivity will greatly decrease.

It is demonstrated that the antibody of the present invention has an equivalent preventive activity against coccidiosis as compared to Salinomycin. Salinomycin is known to have a good preventive activity against coccidiosis. Therefore, it is demonstrated that the antibody of the present invention can protect the uninfected chicks from coccidiosis.

Although Salinomycin is effective as an anti- coccidiosis agent, it is considered to be toxic to human. In contrast, since the antibody of the present invention is a natural product, it is equally effective as an anti- coccidiosis agent but harmless to human.

3. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: This 27th day of November, 2006

Yoshikatsu KODAMA

Review

Poultry coccidiosis: recent advancements in control measures and vaccine development

Rami A Dalloul and Hyun S Lillehoj †

Coccidiosis is recognized as the major parasitic disease of poultry and is caused by the apicomplexan protozoan *Eimeria*. Coccidiosis seriously impairs the growth and feed utilization of infected animals resulting in loss of productivity. Conventional disease control strategies rely heavily on chemoprophylaxis and, to a certain extent, live vaccines. Combined, these factors inflict tremendous economic losses to the world poultry industry in excess of US$3 billion annually. Increasing regulations and bans on the use of anticoccidial drugs coupled with the associated costs in developing new drugs and live vaccines increases the need for the development of novel approaches and alternative control strategies for coccidiosis. This paper aims to review the current progress in understanding the host immune response to *Eimeria* and discuss current and potential strategies being developed for coccidiosis control in poultry.

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Avian coccidiosis is the major parasitic disease of poultry, with substantial economic burden costing the industry an estimated worldwide annual loss of more than US$3 billion [1,2]. In-feed medication for prevention and treatment contributes a major portion of those costs, and losses are also due to mortality, malabsorption, inefficient feed utilization and impaired growth rate in broilers, as well as a temporary reduction of egg production in layers [3]. Good management practices and hygiene help in reducing the spread of coccidiosis, but prophylactic medication and/or vaccination are absolute requirements to control the disease. Coccidiosis is caused by several apicomplexan parasites of the genus *Eimeria* that infect the gut and are transmitted between birds via ingestion of infective oocysts. *Eimeria spp.* possess a complex life cycle, comprising both sexual and asexual stages, are host- and infection site-specific, and their pathogenicity varies in birds of different genetic background [4–7]. Therefore, in the natural host, the immunity is species-specific, such that chickens immune to one species of *Eimeria* are susceptible to others. Understanding the interplay between the host and the parasites in the gut is crucial for the design of novel control approaches against coccidiosis.

Although natural infection with *Eimeria spp.* induces immunity, vaccination procedures on a commercial scale have demonstrated limited effectiveness, and disease control remains largely dependent on routine use of anticoccidial drugs [8,9]. In recent years, several different live vaccines that are composed of either virulent or attenuated coccidian strains have been commercially developed. Major disadvantages of live parasite vaccines are labor-intensive production and high cost due to the inclusion of multiple parasite species in the vaccine [3]. Although live oocyst vaccines represent a limited but useful alternative to prophylactic medication, a recombinant vaccine composed of parasite antigens/antigen-encoding genes that elicit coccidia-specific immunity would be eminently preferable. Altough it may be cost–effective to produce recombinant vaccines (proteins or DNA), the difficulties of identifying the antigens or genes that are responsible for eliciting protective immunity and devising the most efficient delivery

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immunostimulatory effects demonstrated [182,183]. ODN 2006 demonstrated strong stimulatory effects on chicken macrophages, as demonstrated by increased IL-6 secretion, enhanced NO release, upregulated cell-surface marker expression and increased intracellular bacterial killing [183]. In mammalian systems, bacterial DNA displays impressive adjuvant action that influences DNA vaccination. Since their initial discovery [184], CpG ODNs have been demonstrated to play a role in host defense, both by enhancing innate immunity and stimulating T cells and inducing cytokines. The authors have recently used avian-stimulating CpG ODNs to activate chicken innate immunity and enhance protective immune response against *Salmonella* and coccidia. Injection of CpG to 18-day-old embryos enhanced resistance against live coccidia challenge given after hatch [115]. Of four different ODNs tested, two CpG ODNs reduced oocyst shedding, demonstrating that CpG ODNs can amplify protective response when administered with live vaccine. In this regard, when CpG ODN was coinjected with a recombinant microneme protein (MIC2), ODN-injected chickens showed reduction in shed oocysts and improved weight gain. Coadministration of CpG ODN and MIC2 did not have an additive effect in reducing the oocyst output; however, it resulted in the highest and lowest antibody responses before and after *E. tenella* infection, respectively [115]. Taken together, these studies demonstrated that CpG ODNs administered *in ovo* enhance innate immunity following *Eimeria* infections and the feasibility of using CpG ODNs as adjuvants for recombinant vaccines.

Antibody-mediated enhancement of protection against coccidiosis

Immunotherapy using whole antibody molecules or single chain fragments of the variable region (ScFv) with antigen-binding activity has been gaining interest as a potential immunotherapy against infectious agents. The main obstacle to the development of an antibody-based strategy against avian coccidiosis however, is the existence of many different *Eimeria* species. There are potentially two different approaches using antibodies against coccidiosis. One is to produce hyperimmune serum against major immunogenic proteins of coccidia and passively administer it to 18-day-old embryos or to feed it orally to young chicks at hatch. In a recent report, Nguyen and colleagues tested the protective effect of chicken egg antibody (IgY) powder prepared from eggs of laying hens that were hyperimmunized with purified 3-1E recombinant protein in a challenge model with *E. acervulina* and *E. tenella* [185]. Chickens fed standard diet supplemented with IgY powder containing antibodies against 3-1E were better protected against oral challenge with *E. tenella* or *E. acervulina* oocysts compared with those fed standard diet supplemented with IgY-containing powder only. These results clearly indicated that 3-1E represents an important target antigen for coccidiosis prevention, and that passive immunization of chickens with antigen-specific IgY powder is a promising method to confer protection against coccidiosis. Another approach is to develop recombinant antibodies against protective epitopes. The authors have previously produced a ScFv fragment derived from the V_H and V_L genes encoding the 6D-12-G10 monoclonal antibody, which was reactive with an *Eimeria* protein suggested to be involved in binding to a host cell receptor [186]. The ScFv antibody was expressed in *Escherichia coli* and the recombinant gene product bound whole parasites [187] by immunoblot, immunofluorescent assay (IFA) and enzyme-linked immunosorbant assay (ELISA). Chickens fed recombinant ScFv antibodies showed reduced fecal oocysts upon challenge infection with live coccidia [LILLEHOJ HS, UNPUBLISHED DATA]. Using similar approaches, the authors also generated other ScFv antibodies detecting coccidia proteins [188]. Like the native monoclonal antibodies from which they were derived, these recombinant antibodies showed binding activity against *Eimeria* antigens and were secreted at 5 mg/l into culture medium, indicating that soluble, stable and functional chicken ScFv can be produced in large volume. Although the role of antibodies produced during natural infection is debatable, antibodies generated against specific epitope of coccidian parasites can be used to reduce parasite invasion and have been demonstrated to be beneficial against coccidiosis infection [19,185]. The ability to generate an unlimited amount of soluble and functional recombinant ScFv antibodies will facilitate the investigation of their potential therapeutic value in passive immunotherapy against avian coccidiosis. Meanwhile evidence that antibodies in dietary supplements could protect against infection opens a new door for novel immunotherapy strategies against coccidiosis. Moreover, given the limited information concerning the nature of protective antigens of *Eimeria*, these antibodies will be an important tool for affinity isolation of potential *Eimeria* subunit vaccines.

Mushrooms & their extracts protect against poultry coccidiosis

Mushrooms and their extracts have recently gained significant attention in medical research due to their immunoenhancing effects and demonstrated potential in promoting health [189]. Lately, there has been a growing interest in lectins, specifically, largely due to the discovery that some lectins induce various important biological activities including immunomodulation. The authors recently investigated the potential effects of a mushroom lectin (FFrL), extracted from *Fomitella fraxinea*, in inducing immunoprotection in broiler chickens against an *Eimeria* challenge [DALLOUL RA, LILLEHOJ HS, LEE J-S, LEE S-H, CHUNG K-S, UNPUBLISHED DATA]. Growth performance of *E. acervulina*-infected chickens was improved by injecting the FFrL into 18-day-old embryos, as best manifested by higher weight gains over the infected control birds. FFrL-treated chickens also demonstrated reduction in oocyst shedding after infection with live parasites, an indication of improved resistance to coccidiosis. Therefore, this lectin offers a promising means of controlling coccidiosis, especially when coupled with *in ovo* delivery. Other mushrooms and their extracted polysaccharides were also shown to have immunoenhancing potential in chickens and *in vivo* protective effects against *E. tenella* infection [190–192]. These mushrooms and their extracts showed promise in altering bacterial activities and composition in chicken ceca. The polysaccharide extracts showed a slightly significant effect on growth performance, but had no effects on weights of immune and